4 clone 8 cells from $1.6x10^6$ cells/ml stock was added to the wells. The plates were incubated for 1 hr and various concentrations of plant extracts added. After incubation for 5 additional days, the cultures were processed by a modified tetrazolium-based colorimetry (Ayisi et al, 1991). The formula for calculating the percent cell protection from virus induced cell death, inactivity, and/or reduced division has been published (Ayisi et al, 1991). This formula takes into account, the independent effects of the plant extracts on uninfected cells. For determining cytotoxic concentrations from the results in this example, see example 10.

Tables 4 and 5 indicate that plants GHX-2, GHX-6, GHX-7, GHX-26 and GHX-27 were all very effective inhibitors of HIV-1 strain HTLV-IIIB induced cytopathicity. The antiviral indices ranged from 18 to 110 in Molt 4 cells for extracts where 50 percent cytotoxicity values were reached.

Fig. 4 shows by examples, the effects of two of the plant extracts on HIV-1 strain GH3 at four different multiplicities of infection. Plant extracts GHX-2L and GHX-6L had concentration-dependent effects at the four multiplicities of infection and both extracts were able to attain 90% cell protection even at the high multiplicity of infection of 0.11424. For the purpose of comparison, ddAzThd was included in these experiments and proved to be extremely effective except when the multiplicity of infection was increased to 0.11424 where 90% cell protection against GH3 infection was never attained. Thus under the stringent condition of high multiplicity of infection, plants GHX-2 and GHX-6 were more effective than ddAzThd. The effectiveness of the plant extracts were inversely related to the multiplicities of infection. The demonstration of concentration and multiplicity of infection dependent effects clearly indicates that the plant extracts are truly anti-HIV extracts.

5. Effects of period of infection before plant extract treatment on anti-HIV-2 strain GH1 activities.

This example is presented to demonstrate the anti-HIV activities of the plant extracts in preventing or reducing cytopathicity in yet to be acutely infected cells and also in reducing cytopathicity in already acutely infected cultures. The method was similar to the one described in example 4.

Table 6 shows the effects of early initiation of treatment on the antiviral activities of plant extracts and ddAzThd against HIV-2 strain GH1 in Molt 4 clone 8 cells. Plants GHX-2, GHX-6, GHX-7, GHX-26, and GHX-27 had EC50 values that did not elicit any toxicities. Using EC50 values for comparison, ddAzThd and GHX-6 had time dependent decreases in activities (compare Table 6 with Table 7). The time of initiation of plant extract treatment post virus infection had little or no effect on the activities of GHX-2 and GHX-7. When the more pharmacologically accurate EC90 values were used for comparison, ddAzThd was found to be very effective only when treatment was initiated 40 mins after virus infection. Further delay in the start of treatment rendered ddAzThd ineffective. On the other hand, EC90 values were attained for GHX-2, GHX-6, and GHX-7 with minimal cytotoxicities. In fact, the EC90 values for GHX-2 and GHX-6 were markedly reduced to noncytotoxic concentrations of 0.37 and <<0.106 mg/ml respectively when the multiplicity of infection was reduced to 0.006 and treatment started as late as 15 hrs post virus infection (data not shown). Patients who report to hospital with HIV infection, already have the proviral DNA integrated into host cell genome. At 15 hrs in vitro post virus infection, proviral DNA synthesis is completed and integration into host cell genome has already taken place. Thus drugs that are able to inhibit HIV in vitro under such conditions, are likely to have beneficial effects in the treatment of AIDS. The plant extracts by virtue of their effectiveness against HIV in a system where the virus is already incorporated in the genome of the host, should be more effective than classical anti-reverse transcriptase inhibitors in the treatment of clinical AIDS.

6. Effects of plant extracts on virus production in HIV acutely

infected Molt 4 cells.

It was next decided to find out whether the anti-HIV cytopathicity observed for the plant extracts in example 5 was due to actual inhibition of HIV replication and virus particles production. This is important because a reduction in virus load would bear positively on the outcome of treatment of AIDS. GHX-2L and GHX-6L were selected for this experiment because of their high activities against both HIV-1 and HIV-2. Supernatants of HIV-1 or HIV-2 acutely infected Molt 4 cells were titrated in Molt 4 cells. The results in Tables 8 and 9 indicate that both extracts significantly inhibit HIV production in acute infection. Thus the inhibition of cytopathicity seen in the treatment of acute HIV infection in example 4 was likely due to inhibition of virus production.

7. Comparative effects of plant extracts on the replication and viability of Molt-4/HIV (chronically infected) and uninfected Molt-4 cells.

Chronic HIV infection of CD4+ lymphocytes in AIDS may not play a direct role in CD4+ lymphocytopenia. However, these chronically infected cells may serve as factories of HIV production that would cause acute cytopathic infection in uninfected lymphocytes leading to CD4+ lymphocytopenia. A drug may be effective in reducing or stopping this indirect HIV induced CD4+ lymphocytopenia by two different ways.

First, the drug may selectively kill the HIV chronically infected cells at concentrations that have no adverse effects on uninfected cells. Second, the drug may inhibit HIV production from the chronically infected cells. The first mechanism is tested in example 7 and the second mechanism will be described in example 8.

HIV-1 chronically infected Molt-4 cells (Molt-4/HIV) were extensively washed to remove the previously produced viral